

# Monitoring of *Cryptococcus* lung infection with micro-CT and micro-MRI

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## Background

*Cryptococcus* is an encapsulated yeast that causes life-threatening disease in both immuno-competent and immuno-suppressed individuals. The two predominant pathogenic strains of this yeast, *Cryptococcus neoformans* and *Cryptococcus gattii*, are generally found in soil, bird excrement and in the bark of certain trees and enter the host via inhalation. Cryptococcosis mostly affects the lung of a host and may spread to the brain, manifesting itself by cryptococcal meningitis and/or pseudocystic lesions (cryptococcoma) in the brain often with fatal outcome. It remains still unknown why, how and when the cryptococci are able to cross an apparently intact blood-brain barrier (BBB). Histological techniques will remain essential to confirm and unravel cellular and molecular interactions, but imaging techniques are indispensable to define the relevant time frames for histological analysis and this for each animal individually, to investigate crucial events in pathogenesis. As currently imaging tools to evaluate pneumonial cryptococcosis with good temporal and spatial resolution *in vivo* are lacking, advances made in lung  $\mu$ CT and  $\mu$ MRI techniques to follow-up disease progression non-invasively will greatly enhance the cryptococcosis research.

## Aims

It is our objective to dynamically monitor cryptococcosis non-invasively in individual animals, in a mouse model for pneumonial and cerebral cryptococcosis. Thereby, we will establish the kinetics of cryptococcal lung infection with  $\mu$ CT and  $\mu$ MRI and the time profile of *Cryptococcus* spreading to the CNS, thereby defining the most critical time points for histological and immunological analysis of key events in the pathogenesis of cryptococcosis.

## Methods

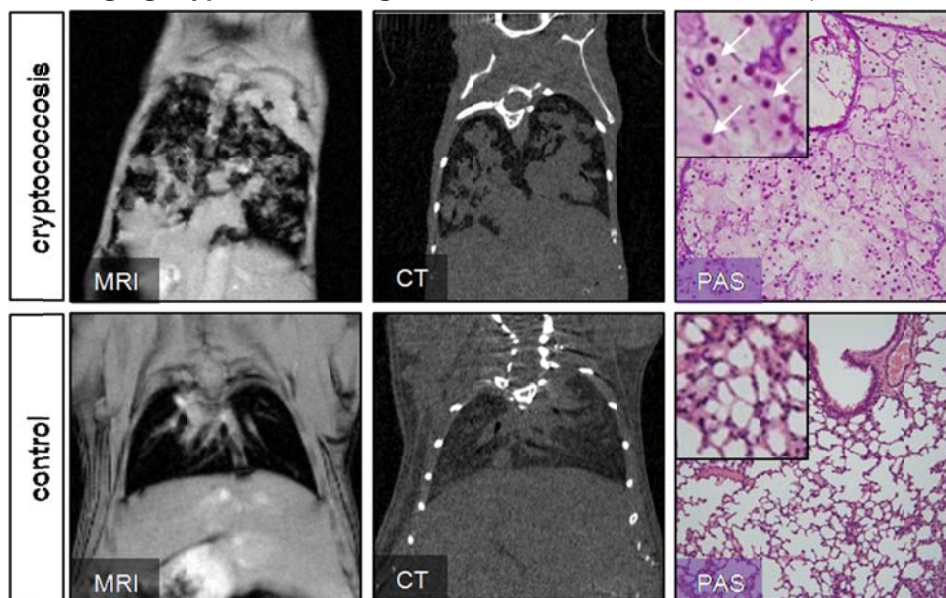
Balb/C mice were infected by inhalation of a *C. gattii* R265 cell suspension (500 cfu's) in PBS (n = 7) or with PBS alone (control, n = 3). Mice were scanned with MRI and CT at baseline and weekly until 45 days post infection. MRI images were acquired on a horizontal Bruker Biospec (9.4T, 20 cm) in combination with a 7cm quadrature coil using a retrospectively gated FLASH sequence IntraGate (Bruker Biospin, Ettlingen, Germany) with the following parameters: TR/TE = 30/1.26 ms, 17 deg flip angle, 5 slices covering the lung, slice thickness 1 mm and gap of 0.5 mm, FOV = 4 cm x 4 cm, matrix 256 x 256, in plane resolution of 156  $\mu$ m, 80 repetitions resulting in a 10 min acquisition; the navigator slab was 1cm wide excited with a 0.8 ms sinc10H pulse with a 1.5 deg flip angle. For reconstruction, 70% of the respiration and ECG period was used (Paravision 5.1, Bruker). CT images were acquired on a dedicated small animal  $\mu$ CT scanner (SkyScan 1076, Kontich, Belgium) with the following parameters: 50 kV, 0.5 mm Al filter, 200  $\mu$ A source current, 35  $\mu$ m isotropic resolution, 120 ms exposure time, 9 projection images per 0.7° rotation step and

retrospectively gated. Image analysis, segmentation and quantification of CT data were performed with custom written algorithms using SkyScan software. After the last time point, mice were sacrificed followed by sterile CNS removal and lung isolation for histological analysis (HE and PAS-stainings) and quantification of fungal load.

## Results

Pneumonial cryptococcosis was successfully and reproducibly induced in immunocompetent mice. While the mice showed no phenotypical signs of cryptococcosis, the progression of the lung pathology could be non-invasively visualized using the here evaluated protocols for IntraGate MRI and  $\mu$ CT at different time points post infection (see figure 1). After optimization of quantification protocols, MRI and CT images were quantified (total lung volume, aerated lung volume ...) and the data correlated for cross-validation. Cryptococcal invasion of the lung and brain (meningitis) was confirmed by histochemical analysis (see figure 1, last panel), lung fungal load was quantified and compared with CT and MRI data.

**Figure 1: Imaging cryptococcal lung infection with MRI and CT.** MRI (first column),  $\mu$ CT (second



column) and histology (PAS-staining, third column) images of a mouse 6 weeks post instillation of a *Cryptococcus* suspension (first row) or PBS (second row). White arrows point at cryptococci in the lung tissue.

## Conclusions & Perspectives

To the best of our knowledge, this is the first study showing that non-invasive monitoring of pneumonial cryptococcosis is feasible with retrospectively gated  $\mu$ MRI (Intragate, Bruker) and  $\mu$ CT resulting in high resolution and contrast images. This imaging approach will allow longitudinal screening of animals, without radiotoxicity concerns when using the MRI, and with even better resolution when using the CT, thereby visualizing infection onset and progression on an individual basis and far before the appearance of any phenotypical signs of disease. We will further finetune the timing of disease onset and correlate this with the time of traversal of *Cryptococcus* cells to the CNS. MR imaging of cryptococcosis will greatly help unraveling the still enigmatic pathogenesis of this life-threatening disease.

**References:**

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